

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicants: Eijiro WATANABE et al.

Serial No.: 08/992,914

Group: 1638

Filed: December 18, 1997

Examiner: D.H.Kruse

For: RAFFINOSE SYNTHASE GENES AND THEIR USE

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of Patents and Trademarks  
Washington, D.C. 20231

Sir:

I, Akitsu NAGASAWA, citizen of Japan and residing in Kamokogahara 3-28-56, Higashi-Nada-ku, Kobe-shi, Hyogo-ken, Japan, declare and say that:

1. I completed the master's course, with a major in agricultural biology, of the graduate school of Kyoto University and obtained a master's degree in agriculture at Kyoto University in March, 1984.

2. From April, 1984 to the present, I have been an employee of Sumitomo Chemical Company, Limited, the assignee of the above-identified application.

3. From April, 1984 to the present, I have been engaged in research works for plant engineering using recombination and other gene manipulation, such as cloning of plant genes, preparation and evaluation of transgenic plants.

4. I am one of the members of the research project related to the above-identified application and am familiar with the subject matter thereof.

5. I have read the Office Action mailed March 11, 2005 and the reference cited, and am familiar with the subject matter thereof.

6. To demonstrate successful identification of raffinose synthase genes in plant, I have made the following computer analysis.

## ANALYSIS

1) The overall sequence homologies (%) among the amino acid sequences of raffinose synthases (RFSs), seed imbibition protein (SIP) and stachyose synthases (STSs) shown in Table 1 attached hereto were calculated based on a global multiple alignment (the alignment of sequences over their entire length) using the gene analysis software GENETYX-SV/RC for Windows version 6.1.0 (GENETYX Corporation; <http://www.sdc.co.jp/genetyx/>) with default parameters. The global multiple alignment was generated using CLUSTAL sequence analysis program. The amino acid sequences of the RFSs, SIP and STSs used to produce the global multiple alignment are as follows:

Sc-02:

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MAPPSITKTATLQDVISTIDIGNGNSPLFSITLDQSRDFLANGHPFLTQV
PPNITTTTTTASSFLNLKSNKDTIPNNNTMLLQQGCFVGFNSTEPKSH
HVVPLGKLKGIKFMSIFRFKVVWTTTHWVGTVNGQELQHETQMLILDKNDSL
GRPYVLLPILENTFRTSLQPLNDHIGMSVESGSTHVTGSSFKACLYIH
LSNDPYSILKEAVKVIQTQLGTFKLEEKTA PSI IDKFGWCTWDAFYLVK
HPKGVWEGVKS LTDGGCPPGFVI IDDGWQSI CHDDDDDDSGMNRTSAGE
QMPCRLVKYEENSKFREYENPENGKKGLGGFVRDLKEEFGSVESVYVWH
ALCGYWGVRPGVHGMPKARVVVPKVSQGLKMTMEDLAVDKIVENGVLV
PPDFAHEMF DGLHSHLESAGIDGVKVDVIHLLELLSEEYGGRVELARAYY
KALTSSVKKHFKGNV IASMEHCNDFLLGTEA ISLGRVGDDFWCSDPSG
DPNGTYWLQGCHMVHCAYNSLWMGNFIQPDWDMFQSTHPCA EFHAASRAI
SGGPIYVSDCVGNHNFKLLKSLVLPDGSILRCQHIALPTRDCLFEDPLHN
GKTMKIWNLNKYTGVLGLFNCQGGGWCPEARRNKSVSEFSRAVTCYASP
EDIEWCNGKTPMSTKGVDFFAVYFFKEKKLR LMKCSDRLKVSLEPFSFEL
MTVSPVKVFSKRFIQFAP IGLVNMLNSGGAIQSLEFDDNASLVKIGVRGC
GEMSVFASEKPVCKIDGVKVKFLYEDKMARVQILWPSSSTLSLVQFLF
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Sc-03:

```
MAPSFSKENSKTCEVANHDDCNTCPIISLEESNFMVNGHVILSQVPSNI
TAISKMGFDGLFVGFD APEPKARHVVS VGQLKGIPFMSIFRFKVVWTTTHW
TGSNGRDLEHETQILILDKSDEGLGRPYIVILPLIEGPFRASLQPGSVDD
YVDICVESGSTKVVGDSFRAVLYIRAGPD PFKLIKDTMKEVQAHLGTFKL
LDDKTTPPGIVDKFGWCTWDAFYLVEXYGVWEGVKGLVENGVPPLVLID
DGWQSI CHDDDPITDQEGINRTSAGEQMPCRLIKYEENFKFRDYKSPNIM
GHEDHPNMGMR AFVRDLKEEFKTVEHVYVWHAFTGYWGVRPNVPGLXEA
QVVTPKLSPGLEMTMEDLAVDKIVNNGIGLVQPDKAQELYEGLHSHLENC
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GIDGVKVDVIHLLEMAEDYGGVELAKTYKAITESVRKHFKGNGVIAS  
MEQCNDFMLLGTETICLGRVGDDFWPTDPSGDIINGTYWLQGCHMVHCAYN  
SLWMGNFIHPDWMFQSTHPCAEFHAASRAISGGPIYVSDVVGKHNIPLL  
KRLVLADGSILRCEYHALPTKDCLFVDPLHDGKTMKIWNLNKYNGVLGV  
FNCQGGGWSRESRKNLCFSEYSKPI SCKTSPKDVWENGHKPFIKGV  
ECFAMYFTKEKKLILSQLSDTIEISLDPFDYELIVVSPMTILPWESIAFAP  
IGLVNMLNAGGAVKSLDISEDNEDKMVQVGIKGAGEMMVSSEKPKACRVN  
GEDMEFEYEESMIKVQVTWNHNSGGFTTVEYLF

Sc-04 (truncated):

MAPSISKVELNSFGLVNGNLPLSITLEGSNFLANGHPFLTEVPENIIVT  
PSPIDAKSSKNEDDDVVGCFVGFHADEPRSRHVASLGKLRGIKFMSIFR  
FKVWWTTHWVGSNGHELEHETQMMLLDKNDQLGRPFVLIPLILQASFRAS  
LQPGLLDDYVDVCMESGSTRVCGSSFGSCLYVHVGHDPYQLLREATKVVRM  
HLGTFKLLEEKTA PVIIDKFGWCTWDAFYLVKHPSGVWEGVKGLVEGGCP  
PGMVLIDDGWQAI CHDEDPITDQEGMKRTSAGEQMP CRLVKLEENYKFRQ  
YCSGKDSEKGMGAFVRDLKEQFRSVEQVYVWHALCGYWGGVRPKVPGMPQ  
AKVVT PKLSNGLKLTMKDLAVDKIVSNGVGLVPPHLAHLLEYGLHSRLES  
AGIDGVKVDVIHLLEMLSEYGGVELAKAYYKALTASVKKHFKGNGVIA  
SMEHCNDFLLGTEAIALGRVGDDFWCTDPSGDPNGTYWLQGCHMVHCAY  
NSLWMGNFIQPDWMFQSTHPCAEFHAPLGPSLVDQFTLVIIVLESTTSSC  
SRASLCLMGRFCVVNTMHS PHETVCLKTPCMMGRQCSKFGISTNIQVFWV  
YLI AKEVGGVP

Sc-05:

MAPPSVIKSDAAVNGIDLSGKPLFRLEGSDLLANGHVVLTDVPVNVTVTA  
SPYLADKDGEPVDASAGSFIGFNLDGEPRSRHVASIGKLRDIRFMSIFRF  
KVWWTTHWVGSKGSDIENETQIIILENSGSGRPYVLLLPLLEG SFRSSFQ  
PGEDDDVAVCVESGSTQVTGSEFRQVYVYHAGDDPFKL VKDAMKVVRVHM  
NTFKLLEEKXPPGIVDKFGWCTWDAFYLTVPDGVHKGVKCLVDGGCPPG  
LVLIDDGWQSIGHDSGDIDVEGMSCTVAGEQMP CRLKFQENFKFRDYVS  
PKDKNEVGMAFVRDLKEEFSTVDYIYVWHALCGYWGGLRPGAPTLPPST  
IVRPELSPGLKLT MQDLAVDKIVDTGIGFVSPD MANEFYGLHSHLQNVG  
IDGVKVDVIHILEMLCEKYGGVRDLAKAYFKALTSSVNKHFDGNGV IASM  
EHCNDFMFLGTEAISLGRVGDDFWCTDPSGDIINGTYWLQGCHMVHCAYNS  
LWMGNFIQPDWMFQSTHPCAEFHAASRAISGGPIYISDCVGQHDFDLLK  
RLVLPDGSILRCEHYALPTRDRLFEDPLHDGKTMKIWNLNKYTGII GAF  
NCQGGGWCRETRRNQCFSQCVNTLTATTNPKDVWNSGNNPISVENVEEF  
ALFLSQSKKLVLSGPNDDLEITLEPFKFELITVSPVVTIEGSSVQFAPIG  
LVNMLNTSGAIRSLVYHEESVEIGVRGAGEFRVYASRK PASCKIDGEVVE  
FGYEESMVMVQVPWSAPEGLSSIKYEF

PsRFS:

MAPPSITKTATQQDVI STVDIGNSPLLSI SLDQSRNFLVNGHPFLTQVPP  
NITTTTTSTPSPFLDFKSNKDTIANNNNTLQQQGC FVG FNTTEAKSHHV  
PLGKLGKIKFTSIFRFKVVWTTTHWVG TNGHELQHETQIL ILDKNISLGRP  
YVLLLP ILENSFRTSLQPGLNDYVDM SVESGSTHVTGSTFKACLYLHLSN  
DPYRLVKEAVKVIQTKLGTFK TLEEKTPPSIIEKFGWCTWDAFYLKVHPK  
GVWEGVKALTDGGCPPGFVIIDDGWQSI SHDDDDPVTERDGMNRTSAGEQ  
MPCRLIKYEENYKFREYENGDNNGGKKGLVGFVRDLKEEFRSVESVYVWHA  
LCGYWGGVRPKVCGMPEAKVVVVKLSPGVKMTMEDLAVDKI VENGVLVP  
PNLAQEMFDGIHSHLESAGIDGVKVDVIHLLELLSEEYGGRVELAKAYYK  
ALTSSVNKHFKGNGV IASMEHCNDFLLGTEAISLGRVGDDFWCCDPSGD  
PNGTYWLQGC HVMHCAYNSLWMGNFIHPDWDMFQSTHPCA EFHAASRAIS  
GGPVYVSDCVGNHNFKLLKSFVLPDGSILRCQHYALPTRDCLFEDPLHNG  
KTM LKIWNLNKYAGVLGLFNCQGGGWPETRRNKSASEFSHAVTCYASPE  
DIEWCNGKTPMDIKGVDVFAVYFFKEKKLSLMKCSDRLEVSLEPFSFELM  
TVSPLKVFSKRLIQFAPIGLVNMLNSGGAVQSLEFDDASLVKIGVRGCG  
ELSVFASEKPVCKIDGVSVEFDYEDKMVRVQILWPGSSTLSLVEFLF

Aj-05:

MAPSFKNGGSNVVSFDGLNDMSSPFAIDGSDFTVNGHSFLSDVPENIVAS  
PSPYTSIDKSPVSVGCFVGFDASEPDSRHVVSIGKLKDIRFMSIFRFKVV  
WTTTHWVGRNGGDLESETQIVILEKSDSGRPYVFLLPIVEGPFRTSIQPGD  
DDFVDVCVESGSSKVVDA SFRSMLYLHAGDDPFALVKEAMKIVRTHLGT  
RLLEEKTPPGIVDKFGWCTWDAFYLT VHPQGVIEGVRHLVDGGCPPGLVL  
IDDGWQSIGHSDPI TKEGMNQTVAGEQMPCRLLKFQENYKFRDYVNPKA  
TGPRAGQKGMKAFIDELKGEFKTVEHVYVWHALCGYWGGLRPQVPGLPEA  
RVIQPVLSPLQMTMEDLAVDKIVLHKVGLVPPEKAEEMEYGLHAHLEKV  
GIDGVKIDVIHLLEMLCEDYGGRVDLAKAYYKAMTKSINKHFKGNGV IAS  
MEHCNDFMFLGTEAISLGRVGDDFWCTDP SGDPNGTFWLQGC HVMHCAND  
SLWMGNFIHPDWDMFQSTHPCA EFHAASRAISGGPIYVSDSVGKHNFDLL  
KKLVLPDGSILRSEYYALPTRDCLFEDPLHNGETMLKIWNLNKFTGVIGA  
FNCQGGGWC RETRRNQCF SQYSKRVT SKTNPKDIEWHSGENPISIEGVKT  
FALYLYQAKKLILSKPSQDL DIALDPFEFELITVSPVTKLIQTSLHFAPI  
GLVNMLNTSGA IQSVDYDDDLSSVEIGVKGCCGEMRVFASKKPRACRIDGE  
DVGFKYDQDQM VVVVQVPWPIDSSSGGISVIEYLF

HvSIP:

MTVTPQITVGDGRLAVRGRTVLSGVPDNVTA AHAAGAGLVDGAFVGATAA  
EAKSHHVFTFGTLRDCRFMCLFRFKLWMTQRMGTSGRDVPLETQFILIE

VPAAAGNDGDSSDGDSEPVYLVMLPLLEGQFRTVLQGNDQDELQICIES  
GDKAVETEQGMNNVYVHAGTNPFDTITQAVKAVEKHTQTFHHREKKTVP  
FVDWFGWCTWDAFYTDVTADGVKQGLRSLAEGGAPPRFLIIDDGWQQIGS  
ENKDDPGVAVQEGAQFASRLTGIRENTKFQSEHNQEETPGLKRLVDETKK  
EHGVKSVYVWHAMAGYWGCVKPSAAGMEHYEPALAYPVQSPGVTGNQPD  
VMDLSVLGLGLVHPRRVHRYDELHAYLAACGVDGVKVDVQNIIVETLGA  
GHGGRVALTRAYHRALEASVARNFDPNGCISCMCHNTDMLYSAQTAVVR  
ASDDFYPRDPASHTVHISSVAYNTLFLGEFMQPDWDMFSLHPAAEYHGA  
ARAIGGCPYVSDKPGNHNFDLLRKLVLPGSVLRAQLPGRPTRDCLFSD  
PARDGASLLKIWNMNCAGVGVFNCQGAGWCRVAKKTRIHDEAPGTLTG  
SVRAEDVEAIAQAAGTGDWGEAVVYAHRADELVRLPRGATLPVTLKRLE  
YELFHVCPVRAVAPGVSFAPIGLLHMFNAGGAVEECTVETGEDGNAVVG  
RVRGCGRFGAYCSRPAKCSVDSADVEFTYDSDTGLVTADVPVPEKEMYR  
CALEIRV

AmSTS:

MAPPYDPIPIPIPM SAILNFLSSTVKDNSFELLDGTL SVKNVPILTDIPS  
NVSFSSFSI VQSSEAPVPLFQRAQSLSSSGGFLGFSQNEPSSRLMNSLG  
KFTDRDFVSIFRKTWWSTQWVGTTGSDIQMETQWIMLDVPEIKSYAVVV  
PIVEGKFRSALFPGKDGHILIGAESGSTKVKTSNFDAIAYVHVSENPYTL  
MRDAYTAVRVHLNTFKLIEEKSAPPLVNKFGWWTWDAFYLTVEPAGIYHG  
VQEFADGGLTPRFLIIDDGWQSINDDNDPNEDAKNLVLGGTQMTARLHR  
LDECEKFRKYKGGMSGPNRPPFDPKKPKLLISKAIEIEVAEKARDKAAQ  
SGVTDLARYEAEIEKLTKELDQMFGGGGEETSSGKSCSSCCKSDNFGMK  
AFTKDLRTNFKGLDDIYVWHALAGAWGGVRPGATHLNAKIVPTNLSPGLD  
GTMTDLAVVKIEGSTGLVDPDQAEDFYDSMHSYLSVGITGVKVDVIHT  
LEYISEDYGGRVELAKAYYKGLSKSLAKNFNGTGLISSMQQCNDFFLLGT  
EQISMGRVGGDFWFQDPNGDPMGVYWLQGVHMIHCAYNSMWMGQFIQPDW  
DMFQSDHPPGGYFHAGSRAICGGPVYVSDSLGGHNFDLLKKLVFNDGTIPK  
CIHFALPTRDCLFKNPLFDSKTI LKIWNFNKYGGVIGAFNCQGAGWDPKE  
QRIKGYSQCYKPLSGSVHVSIGIEFDQKKEASEMGEAEYAVYLSEAELLS  
LATRSDPIKITIQSSTFEIFSFPV IKKLGEGVKFAPIGLTNLFNAGGTI  
QGLVYNELGIAKIEVKGDKFLAYSSVVPKAYVNGAEKVFAWSGNGKLEL  
DITWYEECGGISNVTFVY

PsSTS-1:

MAPPLNSTTSNLIKTESIFDLSEKFKVKGFPLFHDVPENVSFERSFS  
KPSSESNAPPSLLQKVLAYSHKGGFFGFSHETPSDRMLNSIGSFNGKDFLS  
IFRKTWWSTQWIGKSGSDLQMETQWILIEVPETKSYVVIPIIEKCFRS  
ALFPGFNDHVKIIAESGSTKVKESTFNSIAYVHVSFENPYDLMKEAYS  
AIRVHLNSFRLLLEKTI PNLDKFGWCTWDAFYLTVPNIGIFHGLDDFSKGGV

EPRFVIIDDGWQSI SFDGYDPNEDAKNLVLGGEQMSGRLHRFDECYKFRK  
YESGLLLGPNSPPYDPNNFTDLILKGIEHEKLRKKREEAISSKSSDLAEI  
ESKIKKVVEIDDLFGGEQFSSGEKSEMKSEYGLKAFTKDLRTKFKGLDD  
VYVWHALCGAWGGVRPETTHLDTKI VPCKLSPGLDGTMEDLAVVEISKAS  
LGLVHPSQANELYDSMHSYLAESGITGVKVDVIHSLEYVCDEYGGRVDLA  
KVYYEGLTKSIVKNFNGNGMIASMQHCNDDFFLGTKQISMGRVGDDFWFQ  
DPNGDPMGSFWLQGVHMIHCSYNSLWMGQMIQPDWDMFQSDHVC AKFHAG  
SRAICGGPIYVSDNVGSHDFDLIKKLVPDGTIPKCIYFPLPTRDCLFKN  
PLFDHTTVLKIWNFNKYGGVIGAFNCQGAGWDPIMQKFRGFPECYKPIPG  
TVHVTVEWDQKEETSHLGKAEYVYVLNQAEELSMLTKSEPIQFTIQP  
STFELYSFVPVTKLCGGIKFAPIGLTNMFNSGGTVIDLEYVGNGAKIKVK  
GGGSFLAYSSSEPKKFQLNGCEVDFEWLGDGKLCVNPWIEEACGVSDME  
IFF

PsSTS-2:

MAPPLNSTTSNLIKTESIFDLSEKFKVKGFPLFHDVPENVSFERSFSSIC  
KPSESNAAPSLQKVLAYSHKGGFFGFSHETPSDRLMNSLGSFNGKDFLS  
IFRFTWWSTQWIGKSGSDLQMETQWILIEVPETKSYVVIPIIEKCFRS  
ALFPGFNDHVKIIAESGSTKVKESTFNSIAYVHFSENPYDLMKEAYIAIR  
VHLNSFRLLLEKTIPNLVDKFGWCTWDAFYLTVPNPIGIFHGLDDFSKGGV  
EPRFVIIDDGWQSI SFDGCDPNEDAKNLVLGGEQMSGRLHRFDECYKFRK  
YESGLLLGPNSPPYDPKKFTDLILKGIEHEKLRKKREEAISSKSSDLAEI  
ESKIKKVVEIDDLFGGEQFSSVEKSEMKSEYGLKAFTKDLRTKFKGLDD  
VYVWHALCGAWGGVRPETTHLDTKFVPCKLSPGLDGTMEDLAVVEISKAS  
LGLVHPSQANELYDSMHSYLAESGITGVKVDVIHSLEYVCDEYGGRVDLA  
KVYYEGLTKSIVKNFNGNGMIASMQQCNDFFLGTKQISMGRVGDDFWFQ  
DPNGDPMGSFWLQGVHMIHCSYNSLWMGQMIQPDWDMFKSDHVC AKFHAG  
SRAICGGPIYVSDNVGSHDFDLIKKLVPDGTIPKCIYFPLPTRDCLFKN  
PLFDHTTLLKIWNFNKYGGVIGAFNCQGAGWDPIMQKFRGFPECYKPIPG  
TVHVTQVEWDQKEETSHFGKAEYVYVLNQAEECLMTLKSEPIQFTIQP  
STFELYSFVPVTKLCGGIKFAPIGLTNMFNSGGTVIDLEYVGNGAKIKVK  
GGGSFLAYSSSEPKKFQLNGCEVDFEWLGDGKLCVNPWIEEACGV

SaSTS:

MAPPNDPISSIFSPLISVKKDNAFELVGGKLSVKNVPLLSEIPSNTFKS  
FSSICQSSGAPAPLYNRAQSLSNCGGFLGFSQKESADSVTNSLGKFTNRE  
FVSIFRFTWWSTQWVGTSQSDIQMETQWIMLNLPEIKSYAVVPIIVEGK  
FRSALFPGKDGHLISAESGSTCVKTTSFTSIAYVHVSDNPYTLMKDGYT  
AVRVHLDTFKLIEKSAPPLVNKFGWCTWDAFYLTVEPAGIWNKVKEFS  
GGFSPRFLIIDDGWQSI NIDGQDPNEDAKNLVLGGTQMTARLHRFDECEK  
FRKYKGGSMMPKVPYFDPKKPKLLISKAIEIEGVEKARDKAIQSGITDL

SQYEIKLKKLNKELDEMFGGGGNDEKGSSKGCSDCSCKSQNSGMKAFTND  
LRTNFKGLDDIYVWHALAGAWGGVKGATHLNAKIEPCKLSPGLDGTMTD  
LAVVKILEGSI GLVHPDQAEDFYDSMHSYLSKVGITGVKVDVIHTLEYVS  
ENYGGRRVELGKAYYKGLSKSLKKNFNGSGLISSMQQCNDFFLLGTEQISM  
GRVGDDFWFQDPNGDPMGVFWLQGVHMIHCAYNSMWMGQIIHPDWDMFQS  
DHCSAKFHAGSRAICGGPVYVSDSLGGHDFDLLKKLVFNDGTIPKCIHFA  
LPTRDCLFKNPLFDSKITLKIWNFNKYGGVVGAFNCQAGWDPKEQRIKG  
YSECYKPLSGSVHVS DIEWDQKVEATKMGEAEYAVYLTESEKLLLTTP  
SDPIPF TLKSTTFEIFS FVPIKKLGQGVKFAPIGLTNLFNSGGTIQGVVY  
DEGVAKIEVKG DGKFLAYSSSVPKRSYLN GEEVEYKWSGNGKVEVDVPWY  
EECGGISNITFVF

VaSTS:

MAPPNDPVNATLGLEPSEKVFDLSDGKLT VKGVVLLSHVPENVTFSSSFSS  
ICVPRDAPSSILQRVTAASHKGGFLGF SHVSPSDRLINSLGSFRGRNFLS  
IFRKTWWSTQWVGNSGSDLQMETQWILIEVPETESYVVIPIIEKSFRS  
ALHPGSDDHVKICAESGSTQVRASSFGAIA YVHVAETPYNLMREAYSALR  
VHLD SFRLL EETVPRIVDKFGWCTWDAFYLT VNPVG VWHGLKDFSEGGV  
APRFVVIDDGWQSVNFDDDPNEDAKNLVLGGEQMTARLHRFEEGDKFRK  
YQKGLLLGPNAPSFNPETIKELISKGIEAEHLGKQAAASAGGSDLAETIE  
LMIVKVREEIDDLFGGKGKESNESGGCCCKAAECGGMKDFTTDLRTEFKG  
LDDVYVWHALCGGWGVRPGTTHLDSKIIPCKLSPGLVGTMKDLAVDKIV  
EGSIGLVHHPHQANDLYDSMHSYLAQTGVTGVKIDVIHSLEYVCEEYGGRV  
EIAKAYYDGLTNSIIKNFNGSGIIASMQQCNDFFFLGTKQIPFGRVGDDF  
WFQDPNGDPMGVFWLQGVHMIHCSYNSLWMGQIIQPDWDMFQSDHECAKF  
HAGSRAICGGPVYVSDSVGSHDFDLIKKLVFPDGTVPKCIYFPLPTRDCL  
FRNPLFDQKTVLKIWNFNKYGGVIGAFNCQAGWDPKGKKFKGFPECYKA  
ISCTVHVTEVEWDQKKEAEHMGKAEYVYVYLNQAEVLHLMTPVSEPLQLT  
IQPSTFELYNFVPVEKLGSSNIKFAPIGLTNMFNSGGTIQELEYIEKDVK  
VKVKGGGRFLAYSTQSPKKFQLNGSDAAFQWLPDGKLTNLAWIEENDGV  
SDLAIFF

The calculated overall sequence homologies (%) are shown in Table 2 attached hereto. The homologies between RFSs and SIP are less than 40%. The homologies between RFSs and STSs are not higher than 45%. On the other hand, the homologies among RFSs are all 50% or higher. Thus, the homologies among RFSs are higher than those homologies between RFSs and SIP and between RFSs and STSs.

A molecular phylogenic tree of the RFSs, SIP and STSs shown in Table 1 is

drawn in Figure 1 attached hereto. The molecular phylogenetic tree is drawn by the UPGMA method using the gene analysis software GENETYX-SV/RC for Windows version 6.1.0 (GENETYX Corporation; <http://www.sdc.co.jp/genetyx/>) with default parameters. In the molecular phylogenetic tree, RFSs, SIP and STSs form different groups respectively.

In summary, Table 2 and Figure 1 show that RFSs, SIP and STSs can be distinguished from one another based upon a comparison of their amino acid sequences.

2) Attached Table 3 shows the identities obtained using the BLAST program for the amino acid sequences of RFSs, SIP and STSs shown in Table 1. Among Sc-02, Sc-03, Sc-04 and Sc-05, the identities were obtained by searching the "patent database" provided by NCBI (National Center for Biotechnology Information) with default parameters, using the amino acid sequence of each protein as the "query", and using "Protein query vs. translated database (tblastn)" of the NCBI BLAST program. Also, other identities were obtained by searching the "non-redundant database" provided by NCBI with default parameters, using the amino acid sequence of each protein as the "query", and using "Protein-protein BLAST (blastp)" of the NCBI BLAST program. The above-identified amino acid sequences of the RFSs, SIP and STSs are used as the "query" except that the amino acid sequence of Sc-04 used as the "query" is as follows:

Sc-04 (full-length) :

```
MAPSISKTVELNSFGLVNGNLPISITLEGSNFLANGHPFLTEVPENIIVT  
PSPIDAKSSKNEDDDVVGCFVGFHADEPRSRHVASLGKLRGIKFMSIFR  
FKVWWTTHWVGSNGHELEHETQMMLLDKNDQLGRPFVLILPILQASFRAS  
LQPGLLDDYVDVCMESGSTRVCGSSFGSCLYVHVGHDPYQLLREATKVVRM  
HLGTFKLLEEKTAPEVIDKFGWCTWDAFYLVHPSGVWEGVKGLVEGGCP  
PGMVLIDDGWQAI CHDEDPIDTQEGMKRTSAGEQMPCLVKLEENYKFRQ  
YCSGKDSEKMGAFVRDLKEQFRSVEQVYVWHALCGYWGGVRPKVPGMPQ  
AKVVTPKLSNGLKLTMKDLAVDKIVSNGVGLVPPHLAHLLEYGLHSRLES  
AGIDGVKVDVIHLLEMLSEEYGGVELAKAYYKALTASVKKHFKGNQVIA  
SMEHCNDFLLGTEAIALGRVGDDFWCTDPSPGDPNGTYWLQGCCHMVHCAY  
NSLWMGNFIQPDWDMFQSTHPCAEFHAASRAISGGPVYVSDCVGKHNFKL  
LKSLALPDGTILRCQHYALPTRDCLFEDPLHDGKTMKLIWNLNKYTGVLG
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LFNCQGGGWCPVTRRNKSASEFSQTVTCLASPQDIEWSNGKSPICIKGMN  
VFAVYLFKDHKLKLMKASEKLEVSLEPFTFELLTVSPVIVLSKKLIQFAP  
IGLVNMLNTGGAIQSMEFDNHIDVVKIGVRGCGEMKVFASEKPVSCKLDG  
VVVKFDYEDKMLRVQVPWPSASKLSMVEFLF

As shown in Table 3, the identities between RFSs and SIPs are about 40%. The identities between RFSs and STSs range from about 40% to about 50%. On the other hand the identities among RFSs are 60% or higher. The identities among STSs are also 60% or higher. That is, the identities among RFSs or the identities among STSs are higher than the identities between RFSs and SIP or the identities between RFSs and STSs. Thus, RFSs, SIP or STSs can be distinguished based on the results of analysis using BLAST program.

3) Attached Table 4 shows the identities obtained using another BLAST program for the amino acid sequences of RFSs, SIP and STSs shown in Table 1. All possible pair-wised amino acid sequence comparison were made by the "Blast 2 Sequences" program from NCBI (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html>). Sequence identities were calculated using default parameters, program; blastp, matrix; BLOSUM62, open gap penalty; 11, extension gap penalty; 1, gap x\_dropoff; 50, expect; 10.0, and word size; 3. The amino acid sequences of the RFSs, SIP and STSs used to calculate sequence identities are identical to those used as the "query" to obtain identities shown in Table 3. Results were essentially the same with former two types of comparison.

4) In conclusion, raffinose synthases (RFSs), seed imbibition protein (SIP) and stachyose synthases (STSs) were clearly distinguished from one another based on comparison of their amino acid sequences.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonments, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

This 6<sup>th</sup> day of September, 2005

  
Akitsu NAGASAWA

Table 1

Code	Protein*	Organism	Accession**	Reference	Author/Assignee
<b>Sc-03</b>	RFS	<i>Beta vulgaris</i>	E37133	09/301,766	Sumitomo Chemical
<b>Sc-05</b>	RFS	<i>Brassica juncea</i>	E36417	09/301,766	Sumitomo Chemical
<b>Sc-02</b>	RFS	<i>Vicia faba</i>	E24423	08/992,914	Sumitomo Chemical
<b>Sc-04</b>	RFS	<i>Glycine max</i>	E24424	08/992,914	Sumitomo Chemical
<b>Aj-05</b>	RFS	<i>Cucumis sativus</i>	AF073744	Family GH36***	Ohsumi et al.
<b>PsRFS</b>	RFS	<i>Pisum sativum</i>	AJ426475	Family GH36	Peterbauer et al.
<b>HvSIP</b>	SIP	<i>Hordeum vulgare</i>	M77475	Family GH36	Heck et al.
<b>PsSTS-1</b>	STS	<i>Pisum sativum</i>	AJ311087	Family GH36	Peterbauer et al.
<b>PsSTS-2</b>	STS	<i>Pisum sativum</i>	AJ512932	Family GH36	Peterbauer et al.
<b>VaSTS</b>	STS	<i>Vigna angularis</i>	Y19024	Family GH36	Peterbauer et al.
<b>AmSTS</b>	STS	<i>Alonsoa meridionalis</i>	AJ487030	Family GH36	Voitsekhovskaja
<b>SsSTS</b>	STS	<i>Stachys affinis</i>	AJ344091	Family GH36	Pesch and Schmitz

\*Protein: RFS, Raffinose synthase; SIP, Seed Imbibition Protein; STS, Stachyose synthase.

\*\*Accession: GenBank Accession Number.

\*\*\*Family GH36: glycoside hydrolase family 36 (see Carbohydrate-Active Enzymes (CAZy) database: [http://afmb.cnrs-mrs.fr/CAZY/GH\\_36.html](http://afmb.cnrs-mrs.fr/CAZY/GH_36.html))





Table 4

[illegible]

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[GENETYX : Evolutionary tree]
Date : 2004.2.4
Method: UPGMA
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